Evaluation of Larvicidal Properties of Leaves and Root Ethanolic Extracts of Some Plants Herbs against Fourth Instar Mosquito Larvae.

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Abstract- This study was aimed at determining the larvicidal properties of *Balanites aegyptiaca*, *Calotropis procera* and *Eucalyptus globulus* leaves and roots ethanolic extracts against fourth instar mosquito larvae. Twenty batches of fourth instar larvae were exposure to various concentrations of 2,4,6,8 and 10ppm for 24hrs and were assayed according to WHO guidelines for testing laboratory and field mosquito larvicides. Larval mortality was observed after 24hrs and recorded. Results are mean of three replicates. Percentage mortality was calculated using Abbott's formula, while the LC50 was determined by a log dosage probit mortality regression line at 95%CI. One way ANOVA was performed to determine the difference in larval mortality between plant parts. Results obtained shows that *B. aegyptiaca* leaves and roots elicited the highest percentage mortality of 60% and 73% at 10ppm respectively. The lowest mortality was recorded in *E. globulus* leaves and roots as 63% and 64.67% at 10ppm respectively. B. aegyptiaca roots shows a significant difference from the other two plant parts, while values from the leaves are statistically not significant from each other at P<0.05.*B. aegyptiaca* root achieved LC50 at the lowest concentration of 6.91ppm with LCL=4.48 and UCL= 5.31. *E.globulus* leaf achieved LC50 at the highest concentration of 7.94ppm, LCL=4.39 and UCL=5.25. The result of this finding revealed that all the experimental plant parts possessed larvicidal properties at different range. The mortality is concentration dependant; therefore higher mortality can be achieved with higher concentrations. The result of this study concludes that *B. aegyptiaca* root and leaves can be considered to be more potent in terms of larvicidal activities, then *C. procera* and lastly *E.globulus* roots and leaves. Further study will be carried out with increase in number of hours to determine the mortality.

Index Terms- *Balanites aegyptiaca, Calotropis procera, Eucalyptus globulus*, Larvicidal properties, LC50.

I. INTRODUCTION

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis and Japanese encephalitis (Das, 2003). Mosquito alone transmit diseases to more than 700million people annually and the disease are endemic in more than 100 countries(Jang *et al.*,2002).Mosquito in the larval stage are attractive targets for pesticides because they breed in water and thus, are easy to deal with them in this habitat(Nandita,2008).). Continued and repeated use of conventional mosquitocides such as organophosphorus (op) and carbmate insecticides, insect growth regulators and bacterial larvicides has often resulted in the widespread development of resistance and has undesirable effects on non-target organisms. (Rozendal, 1997; WHO, 2006).

Plants are rich source of alternative agents for the control of mosquitoes, because they possess bioactive chemical which act against a number of species including specific target insect and are more environmentally friendly when used in pest control (Lok and Singh, 2003). Mosquitoes control has become increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb the ecological balance. The majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into food chain, and thereby causing an irreversible damage to vital organs. They even result in the mutation of genes and these changes become prominent only after a few generations (Ghosh, 1991). In recent years, use of many synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effects on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem (Brown, 1986). Roark (1947) described approximately 1,200 plants species having potential insecticidal value, while Sukamar *et al.* (1991) listed and discussed 344 plant species that only exhibited mosquitocidal activities. Shaalan *et al.* (2005) reviewed the current state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals and botanical.

Desert date (*Balannites aegyptiaca* (L) Del.), of the family *Balanitaceae*, is indigenous to all dry land south of Sahara and extending southwards (Hall and Walker; 1991). It is an evergreen tree adapted to various climatic conditions especially in arid regions with extremely high temperature and scarce water, thus helps in combating desertification (Gour and Kant, 2012). The tree is widely distributed in many tropical countries of Africa and Asia (Moktar and Abdalla, 2013). The flowering time generally occurs during November-April, while the fruiting takes place during December-July (Moktar and Abdallah, 2013). The tree is

rich is medicinal ingredients and contains other useful products with multi use in rural lives and industry. Among such useful products, high level of oil (30-60%) can be extracted from seeds with valuable application as cooking oil as well as biofuel (Moktar and Abdallah, 2013). Secondary metabolites like rotenone, bergopin, steroids and flavonoids were detected in different parts of the tree (Moktar and Abdallah, 2013). Also, *B. aegyptiaca* is named as an African-Asia saponins producing plant due to its high constituent of saponin compounds.These multiple chemicals have proved different biological activities including moluscicidal, larvicidal, mosquitocidal and insect antifeedant properties, beside other industrial uses (Moktar and Abdallah, 2013). Gajalakshim et al. (2003) reported that B. aegyptiaca contain active component, saponin that prove to have insecticidal properties against *Tribolium castaneum*. Chothani and Vaghasiya (2011) also assert that fruits kernel of *B. aegyptiaca* show larvicidal properties against *Anophelis arabiense* and *Aedes aegypti*. Molluscicidal activities of *B. aegyptiaca* against the snail *Lemnaea acuminata* was also reported by Gajalakshim et al. (2013).

Calotropis procera plant is generally known as milkweed, rubber bush Sodom apple. It is a member of family *Asclepiadaceae* whose members are distributed throughout the world in tropical and sub-tropical regions. It is abundant in warm climate areas having dry, sandy and alkaline soils (Yasin *et al.*, 2012). It is mostly noted in waste and follow lands along roads, streets residential colony parks, sand dines as well as in crop field as weed (Sastry and Kavatheker, 1990). *Calotropis procera* is a soft-wooded, evergreen, perennial shrub. It has one or a few stems, few branches and relatively few leaves, mostly concentrated near the growing tip. The bark is corky surrounded and light gray. A copious white gap shows whenever stems or leaves are cut (Parrotta, 2011). Oyindo et al. (2009) reported that the root of *C. Procera* is helpful in combating headache, malaria fever and convulsion. Latex of *C. Procera* has wound healing activities and anti- diabetic, smooth muscle relevant activity and anti- tussive activity against cough induced bronchi irritation (Rahaman,2012). The pesticidal, antiviral, anti- arthritis laxative properties was also reported by Upaddhyay (2011). The leaves and roots *of C. Procera* contain bioactive components like saponin, alkaloids, phenols, tannins, carbohydrate, terpenoids and flavonoids, which are known to possess medical and pesticidal properties (Azmathullah ,2011).

Eucalyptus globulus was discovered on the island of Tasmania in 1792 by French explores and was one of the first eucalypt species to be formally described. The primeval eucalypt forest of Tasmania was amongst the tallest forest in the world and *E. globulus* tree up to 10lm in higher were recorded (Kumar *et al.*, 2011). By the late 1800's, tress 60-90m high were regularly harvested from south eastern Tasmania and shipped throughout the world for what piles (100-120 feet in length and 20 inches squared). The timber was also in great demand for railway sleepers, street paving blocks and mine supporters (Kumar *et al.*, 2002). Various species of Eucalyptus are cultivated, particularly in sub-tropics and warm temperate regions, on account of their economic value. Eucalyptus species are remarkable for their rapid growth. Some species of them in their natural habitat attain gigantic sizes and are among the tallest trees of the world (Kumer *et al.*, 2011).

Most of the species are popularly called "gum trees" although the exudation from them is not a gum, but an astringent; a tanniferous substance called "Kino". There are over 500 species of Eucalyptus (Kumer *et al.*, 2011). Medicinal plants have been used as a source of remedies since ancient times. (Abu-Shanah *et al.*, 2004). The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in treatment of various disease (Kumer *et al.*, 2011). Kumar et al.(2011) shows that *E. Globulus* has anthelmintic potentials as well as anti bacterial and anti fungal activity. Mozan (1994) also reveals that E. Globulus leaves have potent action against *Culex quinquefasciatus* and *Culex teritaeniorhynhus*. The effects of botanical derivatives against mosquito have been reviewed by Sukumar *et al.* (1991). Extracts from leaves, flowers and roots of plants were found to have mosquito larvicidal activity (Sharma *et al.*, 1998).

II. MATERIALS AND METHOD

2.1 Collection of Plant Materials.

The leaves and roots of *Balanites aegyptiaca.*(Balanitaceae), *Calotropis procera* (*Asclepiadaceae*) and *Eucalyptus globulus* (Myrtaceae) were collected from and around Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria. The plant parts were authenticated by the department of Plant Science of Modibbo Adama University of Technology Yola. Voucher specimens were deposited at the herbarium of the department.

2.2 Preparation of the Stock Solution and Test Concentration.

The leaves and roots of the plant material was shade dried, pulverized and sieved to get a fine powder from which the extract was prepared. Methanol and ethanol extract of the plant were obtained by taking 200mg of the powdered leaves and roots in a separate container and add 20ml of the solvent to it. Screw the cap vial and shake vigorously to dissolve or disperse the material in the solvent. The mixture was then filtered through Whatman filter paper and the filtrate was evaporated under reduced pressure on water bath to obtain the crude extract. The stock solution was serially diluted in ethanol and methanol separately (2ml solution to 18ml solvent). Test concentration was obtained by adding 0.1 – 10ml of the appropriate dilution to 200ml distilled water (WHO, 2005).

2.3 Collection and Rearing of Larvae

Larvae of mosquitoes were collected from any available stagnant water within Moddibo Adama University of Technology and transported to the laboratory of the institution. Larvae was reared in a plastic bowl containing tap water and covered by fine nylon mesh. The larvae were feed with food containing mixture of cabin biscuit and dried yeast until 4th instar larvae was reached. (Adeleke *et al.*, 2008; WHO, 2005)

2.4 Larvicidal Bioassay

The larvicidal activity of the plants crude extracts was assessed according to method recommended by World Health Organization (2005), with slight modifications. 20 fourth instar larvae were transferred by means of a strainer or droppers to small disposable test cups or vessels, each containing 200ml of water. The depth of the water in the cups was maintained between 5cm and 10cm. 0.2ml of the stock was then added to 200ml in the cups to obtain the desired target dosage starting

with the concentrations of 2ppm, 4ppm, 6ppm, 8ppm and 10ppm respectively. Three replicates for each concentration and equal number of control were simultaneously set up with tap water, to which 1ml of the solvent was added. Larval food was then added to each test cup. After 24 hours exposure, larval mortality was then recorded. Moribund larvae were counted as dead larvae for calculating percentage mortality. The result was then recorded on the data recording forms. If the control mortality is between 5% and 20%, the mortalities of the treated groups is then corrected according to Abbott's formula. Corrected Mortality (%) = $\frac{X-Y}{X}$ X 100. Where X= percentage survived in the untreated control and Y percentage survival in the treated sample (Abbott's, 1925).

2.5 Data Analysis

LC_{50} value was calculated from a log dosage – probit mortality regression line at 95%Cl of upper confidence limit (LCL) and lower confidence level (UCL). Using a computer software programme, standard deviation of the mean LC_{50} values were calculated. One way ANOVA was performed to determine the difference in larval mortality between plant parts. Result with P< 0.05 will be considered statistically significant.

Percentage mortality = $\frac{number\ of\ dead\ larvae}{number\ of\ introduced}$ x 100

III. Results

3.1 Larvicidal Activity of Ethanolic Leaf Extracts on Fourth Instar Larvae of Mosquito.

Table 1 and fig.1 shows the percentage mortality of the 4th instar larvae of mosquito exposed for 24hrs to various concentrations of ethanol extracts of the tree plants. The larvicidal activity of *E. globulus* leaves showed, 26.67%, 36.67%, 43.33%, 51.67%, and 63% mortality when exposed to various concentrations of 2,4,6,8 and 10ppm respectively. The 2ppm revealed the lowest mortality of 26.67% of the larvae, however, 63% mortality was observed in 10ppm after 24hrs exposure (fig 1). The *B. aegyptiaca* leaves recorded a result of 28.33%, 36.67%, 48.33%, 51.67% and 66% for the same concentrations of 2,4,6,8 and 10 respectively. Lowest mortality of 28.33% was recorded in 2ppm and the highest mortality of 66% in 10 ppm. Extracts of *C. Procera* showed the lowest mortality of 28.33% at 2ppm while the highest percentage mortality of 64.67% was achieved at 10ppm.

3.2 Larvicidal Activities of Ethanolic Root Extract on Fourth Instar Larvae of Mosquito

Table 2 and fig 2 showed the result of percentage mortality of ethanol extracts of the three experimental plants parts on mosquito larvae. *E. globulus* showed percentage mortalities to be 26.17%, 41.67%, 50%, 51% and 64.67% for concentrations of 2,4,6,8 and 10ppm. *C. procera* showed 38.33%, 48.33%, 50%, 55% and 65% for the same concentrations, while *B. aegyptiaca* root showed 30%, 43.33%, 43.33% 48.33% and 73.67%. Highest mortality was obtained in *B. aegyptiaca* root and the lowest percentage of 64.67% in *E. globulus* at 10ppm. (Fig 2).

3.3 LC50 with fiducial limits (95%) 0f the tested plant extracts against mosquito species.

Table 3: Shows the lethal concentration LC50 with fiducial limit of the tested plant extract against mosquito species. The LC50 value of 7.94ppm with LCL of 4.39 and UCL of 5.25 were recorded for *E. globulus* leaves. In the same way, *B. aegyptiaca* leaves achieved LC50 at 6.70 ppm with LCL of 4.42 and UCL of 5.25, while *C. procera* leaves shows 6.99ppm with LCL of 4.42 and UCL of 5.31. *E. globulus* roots achieved LC50 at 7.24ppm at 4.39LCL and 5.18UCL, *B. aegyptiaca* root showed an LC50 at 6.61 with LCL of 4.48 and UCL of 5.31. *C. procera* root killed 50% at 6.92ppm at LCL of 4.62 and UCL of 5.25.

Table 1 Percentage Mortality of 4th Instar Larvae of Mosquito Species Exposed For 24hrs to Different Concentrations of Ethanol Leaf Extract in ppm.

	Observed Percentage Mortality of Larvae & S.E		
Concentration in ppm	E. globulus leaves	B. aegyptiaca leaves	C. procera leaves
Control	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
2ppm	26. 67 ± 0.89[a]	28.33 ± 0.09[a]	28.33 ± 0.08[a]
4ppm	36.67 ± 0.77[a]	36.67 ± 0.89[a]	41.67 ± 0.70[b]
6ppm	43.33 ± 0.64[a]	48.33 ± 0.89[b]	50.00 ± 0.06[b]
8ppm	51.67 ± 0.09[a]	51.67 ± 0.81[a]	55.00 ± 0.00[b]
10ppm	63.00 ± 1.00[a]	66.00 ± 1.00[a]	64.67 ± 0.09[a]

Values with the same superscript on the same row are not significantly different from each at p<0.05, Values are mean of 3 replicates. (N=3± S.E)

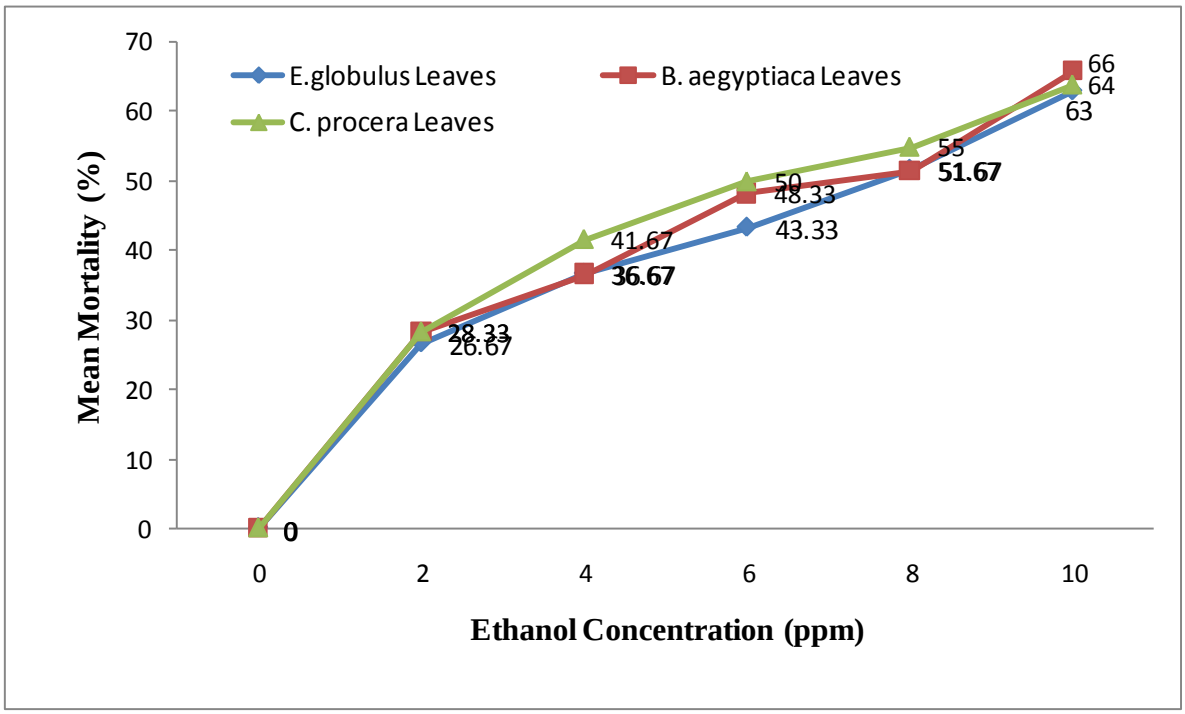

Figure 1 Graph showing the larvicidal activity of *Eucalyptus*, *Balanites*, and *Calotropis* leaves ethanol extracts against the fourth instar larvae of mosquito at different concentration in ppm.

Table 2 Percentage Mortality of 4th Instar Larvae of Mosquito Species Exposed For 24hrs to Different Concentrations of Ethanol Root Extract in ppm

Concentration in ppm	Observed Percentage Mortality of Larvae & S.E. E. globulus root	B. aegyptiaca root	C. procera root
Control	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
2ppm	26.17 ± 1.64[a]	30.00 ± 0.00[b]	38.33 ± 1.00[c]
4ppm	41.67 ± 0.09[a]	43.33 ± 0.19[a]	48.33 ± 0.89[b]
6ppm	50.00 ± 0.00[a]	43.33 ± 0.64[b]	50.00 ± 0.10[a]
8ppm	51.67 ± 0.89[a]	48.33 ± 0.03[b]	55.00 ± 0.00[c]
10ppm	64.67 ± 1.89[a]	73.67 ± 0.08[b]	65.00 ± 0.10[a]

Values with the same superscript on the same row are not significantly different from each at $p<0.05$, Values are mean of 3 replicates. (N=3± S.E)

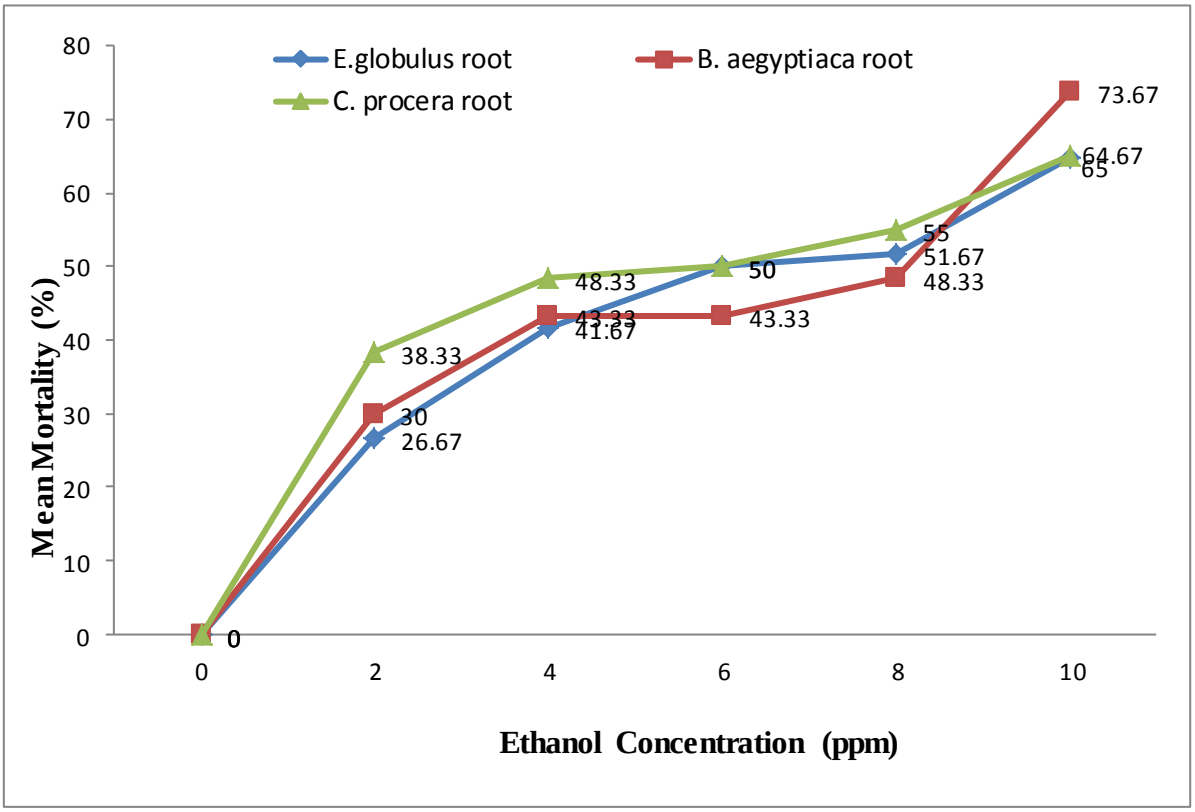

Figure 2 Graph showing the larvicidal activity of *Eucalyptus, Balanites,* and *Calotropis* root ethanol extracts against the fourth instar larvae of mosquito at different concentration in ppm

Table 3 LC50 with fiducial limits (95%) 0f the tested plant extracts against mosquito species.

Plant Extract	LC50(ppm)	95% Confidence limit	
		LCL(ppm)	ULC(ppm)
E. globulus leaves	7.94	4.39	5.25
B. aegyptiaca leaves	6.70	4.42	5.25
C. procera leaves	6.99	4.42	5.31
E. globulus root	7.24	4.39	5.18
B. aegyptiaca root	6.61	4.48	5.31
C. procera root	6.92	4.69	5.25

LC50= Lethal concentration of 50% Mortality. LCL= lower confidence limit, ULC= upper confidence limit, at p< 0.05.

IV. Discussion

Plant bioactive components may serve as a suitable alternative to chemical insecticide as they are relatively safe and available everywhere in the world. The efficacy of botanicals however, generally depends on the plant part (Chapagain and Wiesman,2005), extract concentration, age of plant or location found, solvent used and species of larvae tested. (Babarinde et.al.,2011; Gupta et al.,1990; Olaitan and Abiodun,2011). Most studies report active compounds as steroidal saponins. Saponins are freely soluble in both organic solvents and water, and they work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which is the most probable reason for larval death (Hostettmann et al., 1995). This work reveals the potency of larvicidal properties *of E. globulus*, *B. aegyptiaca* and *C. procera* leaves and roots ethanolic extracts. The results show a dosage dependant pattern by observing high mortality with increase in concentrations. The highest percentage mortality for the leaves of the experimental plants was recorded in *B. aegyptiaca*, values obtained from *E. globulus* and *C. procera* are not significantly different from each other at P<0.05 (fig1). The result of *B. aegyptiaca* leaves was in agreement with the findings of Wiesman et al. (2006) that showed 100% mortality on the larvae of *Aedes aegypti.* The result was also in line with the findings of Monzon *et al. (*1994), that *E. globulus* leaves were found to be the least effective against Aedes aegypti when compared with *Anona squamosa, Azidirachta indica* and

Codiacum variegatum. The ethanolic extracts from the roots against 4th instar larvae exposed for 24hrs reveals that (Table2) *E.globulus* exhibited the lowest percentage mortality, than *C. procera* and the highest mortality was recorded in *B. aegyptiaca*, as compared to all the experimental plants parts. Earlier studies have shown that tissues of B. aegyptiaca plant contains high amount of saponins, therefore the high rate of mortality observed in all tissues of the plant may be attributed to the presence of saponin compound (Liu and Nakanishi,1982; Kamel et al., 1991; Farid et al., 2002). Results obtained from *E. globulus* and *C. procera* are not significantly different from each other at P<0.05. *B. aegyptiaca* root exhibit a significant difference at P<0.05. The LC50 value for *B. aegyptiaca* leaves was lowest with 6.90ppm and that for root is 6.25ppm(Table3). Both the leaves and the root of the experimental plants parts revealed some larvicidal tendencies at different concentration, however, *B. aegyptiaca* root and leave are considered more potent in larvicidal properties compared to the remaining plant parts.

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